

BACTERIUM DISPAR (ANDREWES)

AND ITS ASSOCIATION WITH DYSENTERY:

by

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Thesis submitted for the
Degree of M.D.,

July, 1931.

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INTRODUCTION.

It is common experience in the routine bacteriological investigation of clinical dysentery in the tropics to find frequently, organisms whose morphology, cultivation, characters and biochemistry suggest known groups or even species, but which fail to respond to subsequently applied serological tests in the attempt to identify them.

Many of these recently isolated organisms fail to agglutinate with what appears to be a homologous serum. It is well known that repeated subculture and other manoeuvres directed towards colony selection will render ultimately agglutinable many of these strains. There remains, however, a number of organisms which refuse to disclose their serological identity and remain inagglutinable: such bacteria without antigen overlap or group antigenicity are often found to be species specific and refractory to any but their own sera.

Without discussing the more general and interesting subject of such vagaries in agglutination - much of which has been made easier by the recent work of Arkwright, Bruce White, Landsteiner and others -
it/

it is obvious to all concerned with the bacteriology of dysentery that these inagglutinable bacilli are not now such a problem as in days gone by. The recent study of the Sonne group of intestinal pathogens, which would appear to be serologically homogeneous, has removed from the general mass of inagglutinable bacilli associated with clinical dysentery and described an organism which older methods were slow to recognise as species-specific. *Bacterium dispar*, which would appear to represent not a single serological entity, but a group of organisms, has not been sufficiently studied; its heterogeneity makes its identification more difficult, but it constitutes an important group which, however, up to the present has attracted little attention.

F. W. Andrews, in a communication to the "Lancet" in 1918, described an organism which he called "*Bacillus dispar*;" his article is entitled "Dysentery bacilli; the differentiation of the true dysentery bacilli from allied species."

Since the original description issuing from the pen of Andrews no further adequate investigation appears to have been undertaken, and references to *B. dispar* in the literature are meagre.

In spite of this, a reference in one of our leading/

leading textbooks reads:- "It appears to be a cause of bacillary dysentery in man," while another text-book places it beside B. Sonne and B. Schmitz - apparently as an accepted dysenterogenic organism.

As it belongs to the category of late lactose fermenter, it seems pertinent to explore references to such groups of organisms in which late lactose fermentation is a characteristic. References to B. dispar qua B. dispar are scanty. For these various reasons this Bacterium was chosen as the object of study and forms the subject of this paper.

LITERATURE.

Lembke (1896) isolated a coliform organism producing acid but no gas in glucose and lactose media which he called B. coli anaerogenes.

Sonne (1915) in Denmark differentiated from the rest of the mannite fermenting dysentery organisms a group of slow lactose fermenters having special serological characters.

Thjotta (1919) remarked on the large crenated colonies when grown on litmus agar plates: his strains did not produce indol although apparently they clotted milk.

Andrews (1918): "Dysentery bacilli; the differentiation of the true dysentery bacilli from allied species." The following précis from his paper is descriptive:-

The term *B. dispar* is suggested as a convenient one for the lactose fermenting organism of the dysentery group. It is probable that more than one species comes under this heading as some ferment lactose early, some late. The latter are specially liable to be confounded with Flexner's bacillus. Some of this group are decidedly pathogenic for the rabbit. He gives detail of the technique of acid agglutination and reports *B. dispar* as agglutinating, while Shiga and Flexner bacilli do not. The special characters of *B. dispar* are:- This name is tentatively suggested for the lactose fermenting members of the dysentery group without prejudice as to the number of types or species which may be included. Three strains fermented lactose in 24 hours, others only after 8 to 10 days, and one not until the 24th day. Of eleven strains examined, all fermented glucose, mannite and maltose readily, six fermented saccharose and two fermented dulcitate, in no case with gas production. Indol was formed by four strains only. Litmus milk is acidified and ultimately clotted, but the clotting may/

may not occur for a fortnight or more. In media containing no sugars, alkali formation is as early and vigorous as in the case of *B. Alkalescens*. Agglutination has always been vigorous with Michaelis's test. In its relations with specific Flexner-Y serum it also resembled *B. Alkalescens*. There is no agglutination within the ordinary time limit, but in 20 to 24 hours at 55°C. some clumping may occur in lower dilutions. Even with a serum prepared against itself the poor agglutinability of one strain was manifest in the same way, 24 hours at 55°C. being required. There is no record of the agglutination of these lactose fermenting organisms with the serum of the case from which they have been obtained. Some types at least are pathogenic for the rabbit as already related. The study of the lactose fermenting organisms of the dysentery group appears to be worth while pursuing in fuller detail in relation to human disease.

Pathogenicity of *B. dispar*.

Two strains were tested. The first was a very late lactose fermenter but otherwise gave the cultural reactions of Flexner although agglutinating strongly with Michaelis's test. One c.c. of a formalised/

formalised broth culture injected intravenously into a rabbit killed the animal in less than 24 hours. The small intestine was congested and the contents of the large intestine fluid. The heart's blood was sterile on culture.

A second rabbit was injected with the same formalised broth culture in the attempt to procure an antiserum. Doses of 1/20 c.cm. gradually increasing to 1/2 c.c. were given intravenously and produced no ill-effect. One c.c. was then given subcutaneously when the animal began to lose weight and finally showed paresis of the hind limbs, whereupon it was killed. The titre of the serum of the homologous bacillus was practically nil. A further strain of *B. dispar* was tested on two other rabbits; the first, an animal of 2,060 g., received an intravenous dose of 1 c.c. of a formalised culture which had not been completely killed. No further dose was given. The animal wasted from the first and presently became palsied in the hind legs and then in the forelimbs. It died on the 13th day, the bacillus being recovered from the heart blood. The colon was thin walled and injected and presented doubtful appearances of ulceration. There were no haemorrhages. The second rabbit was treated with small weekly doses of a dead formalinised culture ranging/

ranging from 1/10th to 1/5th c.c. intravenously and finally with doses of one and two c.cs. subcutaneously. It did not waste much and when killed after six weeks yielded a high titre serum. These experiments show that some at least of the late lactose fermenting members of the dysentery group possess considerable pathogenic power for the rabbit. The relation to human dysentery remains unproven for the present. Finally, it is greatly to be desired that in the case of all doubtful pathogenic forms isolated from cases of suspected dysentery that careful observations should be made on the agglutinating power of the patient's serum on the organism isolated as this is an important link in the chain of evidence.

Mita (1921) in Japan described organisms which he called paradysentery A and B, similar to B. Sonne in cultural characters. I have had an opportunity of examining these strains and find they are not antigenetically related to B. dispar but to B. Sonne.

Bamforth (1923), referring to small outbreak of dysentery in an institution, described six cases. In a private communication to me forwarding three cultures he writes:- "I do not think there is any doubt they are Sonne's bacillus." Examined in this laboratory/

laboratory they show no antigenic affinity to *Bact. dispar* and I acknowledge my indebtedness to Dr. Bamforth for his strains.

Nabarro (1923) stated that he isolated *B. coli anaerogenes* from a case of dysentery at Wakefield Asylum in 1912. He further (1927) reported the isolation of late lactose fermenting bacilli from cases of summer diarrhoea and other diarrhoea conditions in children. Later he expressed the belief that *B. coli anaerogenes* and dysentery bacilli of the Sonne type were identical and suggested the name *B. coli dysenteroides* for the group. He described, among other features, dissociated variation in culture, red papillae forming on otherwise white colonies and breeding true.

Referring to Nabarro's observations on dissociated variation, at least five of the strains of *Bact. dispar* under review in this paper show this phenomenon - it would appear to be an obtrusive characteristic of some members of the group - so much so that the name of *Bact. coli anaerogenes mutabilis* might suggest itself.

Nelson (1930), "noting the similarity existing between *E. dysenteries* Sonne, the metadysentery bacilli described by Castellani (1927), and *E. dispar* described/

described by Andrews (1918), cultures of these organisms were obtained from the American Type Culture Collection and were subjected to cultural and serological comparison. Immune serums were prepared in rabbits and cross agglutination reactions carried out. The three organisms have been found to be identical in morphology, in being non-motile, in not liquefying gelatin, in fermenting lactose late, and in rendering milk acid without clot formation. In their fermentation reactions, *E. dysenteriae* Sonne (*Eberthella paradysenteriae* Sonne No. 31) and the metadysentery bacillus (*E. metadysenteriodes* No. 4086) have been practically identical, while *E. dispar* (*Eberthella dispar* No. 29) has been distinctly different in this regard. *E. dispar* has also differed from the other two organisms in producing indol. These results are shown in Table 3. *E. dysenteriae* Sonne and *E. metadysenteriodes* have been agglutinated equally well in immune serums prepared with each of these organisms, while *E. dispar* has not been agglutinated by these serums. Likewise, an immune serum prepared with *E. dispar* failed to agglutinate *E. dysenteriae* Sonne or *E. metadysenteriodes*, but agglutinated the homologous organism in a dilution of 1:5,120. These cross agglutination/

agglutination results are given in Table 4.

"It may be concluded that *E. dysenteriae* Sonne and *E. dysenteriodes* Castellani are strains of the same organism and should not be separately classified, while *E. dispar* Andrewes differs distinctly from these, and consequently is entitled to a place of its own in the late-lactose fermenting group of organisms."

Castellani (1931) includes *B. coli anaerogenes* of Lembke in the metadysentery group along with *B. Sonne* and many others.

Johnston, Marion M. & Brown (1931) "record the characters of 20 strains of the Sonne type of dysentery bacillus, isolated at the Hospital for Sick Children, Toronto. The main interest of their findings, from a bacteriological point of view, lies in their demonstration of a serologically distinct sub-group containing 6 of the 20 strains. This serological differentiation could be demonstrated by direct agglutination as well as by absorption. A serum prepared from a strain obtained from London (Nabarro), and a serum obtained from the Standards Laboratory at Oxford, agglutinated all the strains of the main serological group, but none of the strains of the subgroup. These latter strains could not be differentiated from those of/

of the main group by their fermentation reactions."
(W. W. C. Topley).

It seemed desirable to make some enquiry regarding these strains belonging to the subgroup, and at my request Dr. Johnston sent me representatives of the two serological groups. An accompanying private communication reads:- "You may be interested to know that work completed since the publication of the first paper on the Sonne bacilli and their serological groups seems to show that the differentiation into the two serological groups is based upon the S and R forms of the colonies. The Ridgers group therefore is composed of S type cultures, while the Nabarro group is therefore R in type." Examined in this laboratory, Dr. Johnston's strains showed neither biochemical nor serological equivalence to *Bact. dispar*.

Bergey (3rd Edn.) describes it as "*Shigella dispar* (Andrews) Bergey et al. (*Bacillus dispar* Andrewes, the "*Lancet*," London, 1918, 560; *Bacillus Schmitzii*)."

Topley (*The Principles of Bacteriology and Immunity*) describes *B. Schmitz*:- "Resembles *bact. shigae* except that it forms indol, and produces a slight permanent acidity in milk. It is apparently pathogenic for man, causing bacillary dysentery. No toxin of the shiga type has been reported."

Topley/

Koser et al. (1930) report the result of an examination of 19 strains of *B. Sonne*. They note particularly the absence of indol formation and the reduction of nitrates. A number of other dysentery-like organisms described under a variety of names were found to be identical with the *Sonne* type in every respect. The two *dispar* cultures which were studied in this investigation differed from the *Sonne* strains in their ability to attack xylose and sorbitol and form indol. They were also different serologically. Both types may be readily distinguished from the *Flexner* organisms. They state further that their *Sonne* strains fail to ferment dulcitol.

Bacterium Dispar.

Morphology and Straining Reactions.

The bacillus varies in size from 1.5 to 2.5 μ having parallel sides and rounded ends; its arrangement is irregular, with a suggestion of division to twos, and, though unusual, filaments measuring 10 μ may be encountered in common with other members of the *Bacteriaceae*; coccal forms are sometimes seen. It is non-motile, as shown by repeated examinations of young broth cultures - two-hourly observation for the first six hours followed by a single observation on 48 hours' incubation. This detail in regard to motility is not considered/

considered redundant in the course of routine investigation. In our experience young broth cultures have shown motility to exist in certain intestinal organisms while parallel peptone water cultures have failed to demonstrate it and would suggest that the bacterium was non-motile. A reference to some of the older textbooks will reveal to the reader *Bacillus mobilis* and *Bacillus immobilis* having exactly the same morphology and biochemistry, and in the light of recent knowledge one and the same organism. *Bact. dispar* has not shown motility in any of the strains examined carefully; it is gram negative, non-acidfast and shows neither flagella, spores, capsules, nor irregularity in somatic staining. It has no special morphological character by which it may be distinguished from other members of the genus bacterium.

Cultural characters.

In ordinary broth or peptone water the bacillus grows freely, giving a watered sheen after six hours at 37°C. and general turbidity in an overnight growth. As grown from the stool of a patient on a bile salt MacConkey plate the colonies at the end of 24 hours are large, 1.5 to 2.5 mm. with irregular effuse edge and suggestion of double contour with perhaps a central soupçon/

souppçon of evidence of lactose fermentation at the end of 48 hours; the centre may be definitely red in a colony now measuring 3.5 mm.; the size of these colonies is a characteristic - they are certainly bigger than either Flexner or Shiga - although I have seen colonies of Sonne very similar in size and general appearance.

When picked and grown on agar plates it may show a perfectly round entire edge, but the double contour with more opaque centre becomes more obtrusive on this simple medium. The consistence and emulsifiability differ in no way from other bacteriaceae generally. It is very easy to miss Bact. dispar in the routine examination of MacConkey plates as the colonies may "go over" to acidity by diffusion from surrounding actively fermenting coliform colonies. It is desirable to examine all colonies at the end of 24 hours on this plate and to pick forthwith to another fresh plate for further observation.

In gelatin stab there is after 48 hours on the bench a moderate line of growth confined to the needle track. No liquefaction occurs during three weeks' observation.

Potato yields a vigorous growth. In 48 hours at 37°C. all strains examined produce an abundant cream yellow/

yellow colour.

Media reinforced with blood or serum produce growth without special characters as also does Dorset's egg medium which Andrewes recommended for the preservation of strains.

Resistance (heat).

Six strains grown in nutrient broth and immersed in standard test-tubes for 20 minutes at 60°C. failed to grow on ordinary agar plates after subjection to heat.

These six strains were also grown in brilliant green peptone water using Browning's dilutions. The actual test was carried out by sowing to 10 c.cs. Peptone water tubes having brilliant green dilutions, six Dreyer drops of similar saline opacities (Brown's No.8) of six strains of Bact. dispar and a like number of control strains of B. Coli (Escherich) followed by incubation and reading at 24 and 48 hours.

A sample result of six such experiments:-

	<u>1 - 10,000 B.G.</u>	<u>0.25 c.c.</u>	<u>0.4 c.c.</u>	<u>0.7 c.c.</u>
Bact.dispar -----		+++	++	++
B.Coli (Escherich)-----		++	+	+

Controls of B. Coli Escherich were grown alongside and both/

both plated out in parallel on agar plates. The dispar strains all showed more luxuriant growth than the control organisms. No isolation from faeces either naturally or artificially infected with Bact. dispar through brilliant green was attempted.

Metabolism.

The organism, judged from the behaviour of these six strains, grows best at 37°C. and also at a bench temperature varying between 20 to 25°C. The upper and lower temperature limits of growth have not been determined. Cultures grown overnight on agar slopes and stored in an ordinary dark cupboard are viable six months later. Under anaerobic conditions growth is fair. The power to haemolyse has been carefully studied in view of the importance which some authorities attach to this in organisms of the atypical B. coli group. All strains have been examined against human and sheep's red blood corpuscles both in suspension and on horse blood agar plates all with uniformly negative results.

Fermentation reactions:-

Glucose	}	are uniformly fermented within 24 hours, showing acidity without gas production.
Mannite		
Maltose		

Saccharose. Fermentation usually precedes lactose fermentation (usually 24 to 48 hours).

Lactose. Acidity becomes obvious in the Durham tube at 96 hours, but may appear at 48 hours, or be delayed to 5th or 6th day or even longer.

Dulcitate. Shows irregularity in fermentation.
Some strains show acidity; others do not.

Inosite. }
Salicin. } Are never utilised, and remain unchanged.

Litmus Milk. When compared with the accompanying control it shows acidity on the 4th or 5th day; this is succeeded by clotting about the 7th day; when clotting is completed the litmus is bleached and becomes white. After 15 days it becomes pink from above downwards. Such is the typical reaction in milk.

Peptone Water. A 5 day culture, ether extraction, uniformly gives indol. This positive indol reaction distinguishes *Bact. dispar* from *B. Sonne*.

Nitrates have not been reduced except by one strain (D6) which incidentally ferments dulcitate in 24 hours; repeated observations have been made over varying periods of incubation. Further work is being done on this point.

Neither ammonia (NH_3) nor sulphuretted hydrogen (H_2S) is formed on culture in orthodox media by accepted methods.

Catalase is formed by all strains, while Methylene blue is reduced.

THE METHYL RED (M.R.) test is invariably positive in its swing to the acid side, a further point of distinction from the Sonne group. No power to utilise citrates is demonstrable, while tests for Methyl carbinol yield negative results. On serum or egg or gelatin good growth results without liquefaction over long periods of observation.

Gassul and Zolkovic (1927) found that every bacterial species shows characteristic colour and intensity on illumination with ultra-violet light. Arloing Dolicard and Langeron (1925) attempted differentiation/

differentiation of colonies by ultra-violet light and reported approximate differences.

We have examined, employing Wood's Hanovia quartz cabinet, strains of *B. dispar*, in contrast with *B. coli*, Flexner, and staphylococcus;

- (a) dried bacteria.
- (b) colonies on agar plates.
- (c) peptone water cultures.
- (d) charred bacteria

with entirely negative results in the direction of species differentiation.

Serological considerations.

The strains are simply numbered from D1 to D17, and their serology is now reviewed seriatim; Antisera were prepared as the strains were isolated, first against D1, D3, and D5. All three strains agglutinated to full titre (1__5,000), and they showed complete reciprocal absorption inter se. The American type catalogue states that D5 (D29 of the American catalogue) was originally received from the Lister Institute, London. D3 was sent to me by Dr. Nelson with the information that he got it from the American collection, being D29 American catalogue.

So/

So D1 B. dispar Andrewes 1602 Nat. type culture.

D3 (Nelson).

D5 (D29 American type culture)

are probably one and the same strain.

An antiserum was then prepared against D2 (Fikri); this strain was isolated in Cairo and is homologous in agglutination and absorption with the three foregoing strains; its biochemistry is detailed later on.

D4 (21,508) isolated Cairo, and on isolation inagglutinable with D1 serum, subsequently agglutinated after frequent subculture to one tenth of the titre of that serum; an antiserum prepared against it will not agglutinate D1, D2, D3, or D5, but does agglutinate D9 to full titre.

D9, (21,579) isolated Cairo, appears to be the same organism serologically as D4. They show complete reciprocal absorption of agglutinins.

D6 (American D30) agglutinated with D1 serum (1__5,000) and with incomplete reciprocal absorption of agglutinins.

D7 (1908) isolated Cairo. After repeated subculture it does not agglutinate with any of the foregoing sera; an antiserum/

antiserum prepared from it will not agglutinate D1, D2, D3, D4, D5, or D6, but does agglutinate D8.

D8, (1897) isolated Cairo, is homologous with D7.

D10 (Hulke); D11 (Lewis); D12 (Clelland); D13 (Davis) appear not to be serologically related to any of the foregoing or to one another, and they would appear to represent four heterogeneous strains.

D14 (Cromer) serum prepared against this strain will not agglutinate any of the foregoing strains or those to follow, D15, D16, D17.

D15 (Shaker 3) proved to be a poor antigen, and four injections to rabbit ($\frac{1}{2}$ c.c. and 1 c.c. subcutaneously followed by $\frac{3}{4}$ c.c. and $1\frac{1}{4}$ c.c. killed agar slope culture) failed to produce agglutinins in rabbit; work to be repeated. The strain is not itself agglutinated by any of the previous sera.

D16 (Shaker 4). An antiserum was prepared against this strain and it was found serologically different from all strains D1 to D15, but agglutinated to full titre with also complete reciprocity in absorption the strain which follows, D17 (Brown) isolated some weeks before.

D17 (Brown) is biochemically and serologically the same organism as D16.

It was thought desirable, in the quest for strains of *B. dispar*, to explore the agglutination level of sera sent to the clinical laboratory for the Wassermann reaction, and possibly pick out a few showing sufficiently high titre to warrant further investigation, e.g., examination of stools, &c. Strains D1, D4 and D7 were used, and it was found that no serum agglutinated any of these strains beyond 1 in 25.

Three years ago also examining 400 sera in the same way against the Flexner group it was not unusual to pick out sera agglutinating at 1 in 125 (Dreyer technique), but this has not been so with the *dispar* strains, and in a country like Egypt is of very considerable importance. Serological relationship to the Sonne and Flexner groups is shown by the following table. Four *dispar* sera (1-5,000) prepared by immunising rabbits with strains D1, D2, D4 and D7 behave as follows:-

	B.Flexner.	B.Sonne.	B.Sonne.	B. <i>dispar</i> .
	vwxyz. Oxford. St.C.	(Lister)	Oxford. St.C.	
Dispar serum.	1.25	1.25	1-50	1-5,000
Sonne serum Oxford 1/250S.	Nil.	1/250S	1/250	Nil.
Flexner Polyvalent (Oxford).	Standard Agglutination.	Nil.	Nil.	Nil.

Pathogenicity.

Rabbits have been fed on this organism without showing evidence of infection. Young, recently-isolated strains have been introduced by rubber catheter to rabbits' rectum without harmful result to the animals. A monkey (*macacus rhesus*) was fed in my room each morning on vegetable impregnated with young broth culture and by sugar soaked in the culture, before other food was allowed each day. This continued for a fortnight without signs of enteritis intervening. The monkey appeared happy and undisturbed throughout the month of experiment. The stools of the animal were sown on three occasions on MacConkey's medium and yielded large crops of colonies of *B. dispar*. Any deduction from this however must be made with caution as I have repeatedly fed monkeys in India on recently isolated and virulent dysentery bacilli without inducing true dysentery. At the same time this negative result is interesting. The rest of the story, however, is important, and here it is: A laboratory attendant in charge of the monkey, the second day of the feeding it, himself ate the cabbage stump heavily impregnated with young broth culture of strain D2 (Fikri) to my intense joy and delight. No one had asked him to participate in the monkey's diet and/

and the illness ahead of him was entirely his own affair! Ten days went by and nothing happened - monkey and attendant alike thrived on B. dispar until fourteen days from date of pecculation when Abdul Hamid went down with an acute attack of dysentery, having elevated temperature, malaise and blood and mucus in his stool. Our hopes were high, but doomed to disappointment. Flexner V was isolated from his stools, 40 non-lactose fermenting colonies were examined for dispar - unsuccessfully. None could be found in a keen search. His blood later agglutinated Flexner V, Oxford, 1-250, and his own Flexner bacillus later on (1-125) and left B. dispar "disinterested." It is probable he got his infection while cleaning dishes in the laboratory kitchen.

Enterotropism in the rabbit.

Some observers have by parenteral, usually intravenous, introduction of living culture, shown selective inflammation of the mucosa of the large intestine in this animal. It will be admitted by those who have had considerable experience of this manoeuvre that enteritis does not always follow injection of what is undoubtedly living dysentery antigen. Personally I find it is more usual to kill the rabbit with post-mortem evidence of acute toxæmia and without lesion referable/

referable to the intestinal mucosa. It is not rare to find both ureters of the animal ballooned with blood produced by an acute haemorrhagic nephritis. We do admit, however, that certain strains of Flexner may in very small doses produce on occasion enterotropism of the large intestine, as evidenced by the accumulation of blood and mucus therein and the presence of small ulcers or areas of haemorrhagic usually large but occasionally small intestine. In regard to *B. dispar* I have not been able to produce in rabbits evidence of inflammation of the lower gut. Here is an example of an experiment we have carried out and repeated with six strains of the organism. Three rabbits A, B, C, were intravenously injected with 1/6th, 2/6th, 3/6th of an agar slope in saline. C, receiving the largest dose, died in 24 hours. Post-mortem, no obvious bowel lesion was visible or other suggestion of enterotropism except acute congestion of the first 5 cms. of duodenum; serious effusion to peritoneal cavity was present and cultures from heart blood, gall-bladder, spleen, and peritoneal fluid produced pure growth of *B. dispar*, whereas the growth from the mid and lower intestine was almost wholly of lactose fermenting colonies. Rabbit B died in 48 hours; no macroscopic change in intestine except/

except as before in rabbit C the duodenum showed injection of its mucosa and contained bile-stained bloody mucus, while the gall-bladder was greatly distended. Smears from heart, gall-bladder, and spleen yielded pure cultures of the infecting organism. Sowings from upper, mid, and lower intestine yielded chiefly from each, non-lactose fermenting *dispar* colonies.

Rabbit A died within 72 hours.

As before, heart blood, peritoneal fluid, gall-bladder, and spleen all gave pure cultures of *B. dispar*.

MacConkey plates from intestine showed:-

- (a) From upper gut sowings *dispar* colonies were recovered.
- (b) From mid-gut sowings *dispar* colonies still more abundant.
- (c) While from lower-gut sowings few *dispar* colonies were recoverable, the large majority being lactose fermenters.

Further, we have failed to produce enterotropism with this bacillus using 1/10th to 1/4 mg. per kilo. intravenously.

B. dispar is toxic to rabbits, as pointed out by Andrewes. We have found the M.L.D. somewhere between one and two mgs. per kilo., without employing a large number/

number of animals expressly for this determination.

In small doses it is an efficient antigen, and no difficulty has been found in obtaining from rabbits sera having a titre of 1:5,000. A vaccine containing 20 mills. per c.c. was prepared and used at my request by the Professor of Clinical Medicine (Major A. G. Biggam, R.A.M.C.) to treat by protein shock a drug addict (heroin) in his wards. The patient's serum, after seven or eight inoculations, had a titre of 1 in 500, ascending doses 0.1 to 1.5 c.c. being used.

The organism does not produce a soluble exotoxin as shown by the intravenous injection of 5 c.c. of 12 hour growth phase bacterium free and sterile filtrate drawn through a Seitz filter. The animal was off its feed for 24 hours, but was alive at the end of a month.

DISCUSSION.

In our present state of knowledge there can be no objection to Bact. dispar being regarded as belonging to the broader and undefined B. coli anaerogenes group of bacteria in view of morphological and biochemical equivalence. Many of the organisms of this group ferment lactose in 24 hours without gas and attract no attention by our ordinary methods of routine study. Some of the bacteria in the group show varying ability to utilise lactose, and those that take more than 24 hours to produce evidence of doing so have been called late lactose fermenters. It would appear that some strains are serologically homogeneous within the group while others having an exactly similar biochemistry will not agglutinate with a common serum, nor do they produce a serum which agglutinates a standard dispar culture. In the early stages of this investigation some of the strains were found to be serologically homogeneous, and it was imagined that B. dispar might be a pathogen whose strains were alike in their behaviour to an antiserum. Further work, however, showed that this is not so.

In/

In a survey of students' stools (86 examined), each to four 10 cm. plates, we did not once isolate *Bact. dispar*.

During this winter I have isolated seven strains of *B. Asiaticus* from the stools of Britishers resident in Cairo, most of them from samples of faeces given me by Dr Robert Brown, and all of them collected from cases of chronic "enteritis." Each strain isolated was examined against the serum of the patient and in every case with negative result. Doubtless this particular organism has been described as bacteriaemic and has on occasion been isolated by blood culture, and because of this accident fevers have been ascribed to it in the literature. Personally I am not convinced it is a pathogen when isolated from the stool, and nothing short of a rising titre in the serum would suggest to me it can produce a specific fever and cause disease.

The case of *Bact. dispar* is exactly similar; it is curious that all the strains in this investigation are from cases of dysentery and in many of them already an accepted causal organism had been found. In none was there evidence of parenteral invasion in the absence of agglutinins or other serum indicators. It is the experience of everyone engaged in the routine examination/

examination of stools from cases of enteritis and dysentery to find a disturbed flora. It is not unusual to pick as many as ten non-lactose fermenters from a single plate and to find that further procedure identifies these as late lactose fermenters producing acid and gas, and in this search to come across such organisms as *B. alkaligenes*, *B. Morgan*, and, I would add, *Bact. dispar*.

Each of these biological entities has a place in the normal intestinal flora, but only a very small and limited one. Occupying such a place, they are known as commensals, and not usually isolable by the ordinary methods. When, however, a pathogen comes amongst them and multiplies, they too appear to increase in numbers, a symbiosis is suggested, and they become known as concomitants.

CONCLUSION.

Bact. dispar (Andrewes) would appear to belong to the larger group of organisms described as B. Coli Anaerogenes. It is probably a commensal which becomes a concomitant when the normal flora of the bowel is disturbed by the entry of a bowel pathogen. It cannot be accepted as a dysenterogenic organism until further serological evidence is obtained from the study of many strains.

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Strain.	Origin and Clinical Data.	Acid in -										Indol.	M.R.	V.P.	Nitrates.	Catalase.	H ₂ S.	Gelatin.	Serology.
		Motility.	Lactose.	Glucose.	Mannite.	Dulcite.	Saccharose.	Salicin.	Maltose.	Inosite.	Litmus Milk.								
D ₁	B. dispar (Andrewes) 1602. Nat. type culture.	O	A	A	A	O	A	O	A	O	A.C.	+	+	O	O	+	O	O	Antiserum prepared from rabbit titre (1-5,000) = Antiserum D ₁ .
D ₂	Strain "Fikri" isolated from case of enteritis; from same case Flexner Y was isolated.	O	A	A	A	O	A	O	A	O	A.C.	+	+	O	O	+	O	O	(a) Patient's serum agglutinates Flexner Y (Oxford St. Agg. Cult.) to titre (1-250). (b) Patient's serum does not agglutinate own Dispar or D ₁ . (c) Str. Fikri agglutinates with Antiserum D ₁ to titre. (d) Complete reciprocal absorption of agglutinins.
D ₃	Strain "Nelson" being No. 29 of American type Collection and originally received from Lister Institute.	O	A	A	A	O	A	O	A	O	A.C.	+	+	O	O	+	O	O	Serologically identical with B. dispar (Andrewes) D ₁ .
D ₄	Strain "(21508)" isolated from case of amoebic dysentery, Cairo. No other pathogen isolated.	O	A	A	A	O	A	O	A	O	A.C.	+	+	O	O	+	O	O	(a) Patient's serum does not agglutinate even in low dilutions. (b) Agglutination with anti-serum D ₁ (1-500). (c) Serologically identical with D ₉ "(21579)". (d) Antiserum D ₄ will not agglutinate D ₁ , D ₂ , D ₃ , or D ₅ .
D ₉	Strain "(21579)" isolated from case of "chronic intestinal trouble" - diarrhoea, &c.	O	A	A	A	O	A	O	A	O	A.C.	+	+	O	O	+	O	O	(a) Patient's serum does not agglutinate this organism. (b) Serologically identical with D ₄ "(21508)".
D ₅	Strain D ₂₉ American type Catalogue. Received direct from U.S.A.	O	A	A	A	O	A	O	A	O	A.C.	+	+	O	O	+	O	O	Serologically identical with D ₃ Nelson.
D ₆	Strain 30, American type Catalogue. Received direct from U.S.A.	O	A	A	A	+						+	+	O	Red ⁺	+	O	O	Incomplete reciprocal absorption with D ₁ .
D ₁₀	Strain "Hulke," isolated from Dr. Hulke, Cairo, suffering from repeated attacks of dysentery.	O	A	A	A	O	A	O	A	O	A.C.	+	+	O	O	+	O	O	(a) Patient's serum does not agglutinate own Dispar. (b) Patient's serum does agglutinate B. Sonne Oxford St. Culture (1-250). Also a Lister strain of B. Sonne (1-125). (c) It does not appear to be serologically related to any other B. dispar strain isolated.
D ₇	Strain "(1908)". Isolated Cairo from case of renal colic who developed diarrhoea while in hospital; no blood or mucus; several dispar colonies on plates.	O	A	A	A	O	A	O	A	O	A.C.	+	+	O	O	+	O	O	(a) Patient's serum does not agglutinate it. (b) Serologically distinct from any of these dispar strains except D ₈ "(1857)" with which it is homologous and shows complete reciprocity in agglutinin absorption.

Table (Contd).

Strain.	Origin and Clinical Data.	Acid in:-										Indol.	M.R.	V.P.	Nitrates.	Catalase.	H ₂ S.	Gelatin.	Serology.
		Motility.	Lactose.	Glucose.	Mannite.	Dulcitol.	Saccharose.	Gallicin.	Maltose.	Inositol.	Litmus Milk.								
D ₈	Strain "(1897)" isolated from case of dysentery which showed also B.Sonne.	O	A	A	A	O	A	O	A	O	A	C.	+	O	O	+	O	O	(a) No agglutination with patient's serum. (b) B.Sonne (Oxford St.Aggs. Cult.) does agglutinate with patient's serum (1-125). (c) Patient's serum will not agglutinate any dispar strain.
D ₁₂	Strain "Clelland" isolated Cairo from case of dysentery (Dr Pochin) boy of 4 years. B.Sonne also isolated. Recurrent attacks of dysentery.	O	A	A	A	+	A	O	A	O	A	C.	+	O	O	+	O	O	(a) No serum of patient available. (b) Serologically distinct from other strains collected.
D ₁₁	Strain Lewis: received from P.H.Lab., Cairo; from clinical dysentery stool.	O	A	A	A	O	A	O	A	O	A	C.	+	O	O	+	O	O	(a) Patient's serum not available. (b) Does not agglutinate with any dispar serum. (c) Appears to be serologically distinct.
D ₁₃	Strain "Davis;" isolated by Dr. Davis, chief bacteriologist P.H.Lab., Cairo, from his own stool while suffering from dysentery; B. Sonne also isolated by himself at same time.	O	A	A	A	A	A	O	A	O	A	C.	+	O	O	+	O	O	(a) His serum agglutinates B.Sonne, Oxford, to titre also his own B.Sonne isolated. (b) It does not agglutinate B.dispar isolated, nor any dispar strain examined against it. (c) This strain would appear to be serologically distinct.
D ₁₄	"Cromer" 17 strain from child clinical dysentery. B.Flexner Y also isolated at same time.	O	A	A	A	A	A	O	A	O	A	C.	+	O	O	+	O	O	(a) Patient's serum not available. (b) Organism serologically distinct.
D ₁₅	Strain 3 Shaker, isolated from case of clinical dysentery.	O	A	A	A	O	A	O	A	O	A	C.	+	O	O	+	O	O	(a) Patient's serum not available. (b) Appears to be serologically distinct.
D ₁₆	Strain 4 Shaker, isolated from case of clinical dysentery.	O	A	A	A	O	A	O	A	O	A	C.	+	O	O	+	O	O	(a) Patient's serum not available. (b) Serologically identical with D ₁₇ .
D ₁₇	Strain Brown isolated from case of colitis of indefinite origin and of several weeks' duration.	O	A	A	A	O	A	O	A	O	A	C.	+	O	O	+	O	O	(a) Patient's serum does not agglutinate this organism. (b) Serologically identical with D ₁₆ .

To The Dean of the Medical Faculty
University of Glasgow.

Sir,

I beg to submit attached thesis
for consideration for the degree of M.D.;
further that it be considered for the
Stuart Settlements Gold Medal which
according to the Univ. Calendar is allotted
to Glasgow this year.

W. Leonard Forsyth.

L.B.S. Glas.
Commendⁿ - 1908)
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July 10th 1931.

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